

U.S. Appln. No. 09/476,485
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Comm. Resp. to Examiner Inquiry dated November 22, 2004

EXHIBIT I

A copy of pages 120-121 of the instant application which provides the amino acid sequence of Yam-FRIL.

EXAMPLE 22Purification and Cloning of YamFRIL from *Sphenostylis stenocarpa*

Dry seeds of Yam bean (*Sphenostylis stenocarpa*) were ground in a coffee mill and the powder was extracted with 5 volumes of 10 mM Na Acetate buffer, pH 5.2, containing 1 mM CaCl₂ for 1 hour at 4°C. After centrifugation, the clear supernatant was neutralized with Tris-HCl pH 8.0.

YamFril purification was achieved through and absorption on a ovalbumin gel affinity column (Sigma) and was eluted with 200 mM trehalose.

The resulting protein was fractionated into 2 polypeptides that were submitted to N-terminal amino acid sequences.

Beta band: AQSVSFTFTKFDSDQ (SEQ ID NO: 9)

Alpha band: AASNNVVAVEFDTXPN (SEQ ID NO: 10)

Reverse-transcriptase PCR was performed on total RNA obtained from International Institute of Tropical Agriculture (Ibadan, Oyo State, Nigeria), using degenerate primers based on the alpha and beta N-terminus sequences (i.e., SEQ ID NO: 10 and SEQ ID NO: 9, respectively).

3' RACE PCR was performed on total and polyA+ RNA using gene specific primers with an Anchor Primer.

Partial cDNA clones were obtained and the following sequences deduced.

YamFril: partial mRNA sequence

ACGAAGTTGACAGCGACCAAAAGGATCTTATGTTCCAAGGTCAACCATTCTAGCAGC
25 AATGTCATACAACTCACCAAGTTAGACAGTAATGGAAACCTGTGAGTACCAGTGTGGGA
AGAGTGTATACTCTGCACCATTGCGCCTTGGGAAAGCTCTACAGTAGTGTCAACCTT
GAGACCACCTTCACCTTCAAATCTCAACACCTTACACTAGTCCTCTGGTGTGGCTC
GCCTTCFTCCTTGCACCATATGACACTGTCACTCCCTCCAAATTCTGCTGGCAATCTTCTT
GGACTCTTCTAACTTAAATGCTTTAAGAAACTCCACCAACCAGTAAAGAAACCACTATT
30 GATGTCAATGCTGCATCTAACAAACGTTGTTGCCGTTGAATTGACACCTACCCCTAACGAC

AATATTGGTGATCCAAGATACAAACACATTGGAATCGATGTCAACTCTATCAGGTCCAAG
GCAACTGTTGCGTGGGACTGGCAAAATGGGAAAACAGCCACTGCACACATCAGCTATAAC
TCTGCCTCTAAAAGACTATCTGTTACTACTTTTATCCTGGGGTAAAGCTGTGAGTCTT
TCCCATGACGTTGAGCTCACTCAAGTGCTCCTCAATGGATTAGAGTAGGGTTCTGCT

5 TCAACAGGATTAGAGAAA (SEQ ID NO: 7)

YamFrl: deduced amino acid sequence

AQSVSFTPTKFDSDQKDLMFQGHTISSNVQLTKLDSNGNPVSTSVGRVLYSAPRLWE
SSTVVSTFETTFQ1STPYTSPPGDGLAFFLAPYDTVIPPNSAGNLLGLFPNLNALRNS
10 TTSKETTIDVNAASNNVVAVEFDTYPNDNIGDPRYKHIGIDVNSIRSKATVAWDWQNGKT
ATAHISYNSASKRLSVTTFYPGGKAVSLSHDVELTQVLPQWIRVGFSASTGLEK (SEQ ID
NO: 8)